THE MECHANISM OF CARBOHYDRATE OXIDATION: THE ACTION OF COPPER ACETATE SOLUTIONS ON FRUCTOSE

STANFORD UNIVERSITY LIBRARY
APR 27 1929

DISSERTATION

PRESENTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN THE GRADUATE SCHOOL OF THE OHIO STATE UNIVERSITY

BY

CHARLES EDWARD WARING, A.B., M.A.

STANFORD UNIVERSITY LIBRARIES
CHEMISTRY

The Ohio State University

1927

3781
937W

UNCLASSED
Dissertation

THE MECHANISM OF CARBOHYDRATE OXIDATION: THE ACTION OF COPPER ACETATE SOLUTIONS ON FRUCTOSE

DISSERTATION

PRESENTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN THE GRADUATE SCHOOL OF THE OHIO STATE UNIVERSITY

BY

CHARLES EDWARD WARING, A.B., M.A.

The Ohio State University

1927

PREFACE

The work herein described was undertaken at the suggestion of Dr. William Lloyd Evans, and carried out under his direction. Many thanks are due him for his interest and enthusiasm in the work. Thanks are also due to the E. I. du Pont de Nemours & Company for financial assistance in the form of a Fellowship while this work was in progress.

TABLE OF CONTENTS

	PAGE
Introduction	5
Experimental Part	5
Theoretical Part	12
Summary	17
Autobiography	19

THE MECHANISM OF CARBOHYDRATE OXIDATION. THE ACTION OF COPPER ACETATE SOLUTIONS ON FRUCTOSE

Introduction

The mechanisms proposed in some of the more recent literature dealing with the oxidation of carbohydrates in alkaline solutions rest on one or more of the following ideas: (*a*) that carbohydrates in alkaline solutions are converted into equilibrated systems consisting of a number of isomeric carbohydrates;[1] (*b*) that these carbohydrates give rise to a series of three enediols which form a restricted equilibrium;[2] (*c*) that the general equilibrium consisting of the components in (*a*) and (*b*) may be influenced by certain experimental factors, such as a change in both the temperature of the reaction and also the concentration of the alkali used.[3] Nef[4] states that these enediols do not exist in solutions of an alkalinity less than $N/30$, and that they begin to decompose in systems above an alkalinity of $N/20$. In view of the data obtained in this Laboratory with reference to the behavior of glucose, mannose and fructose toward aqueous solutions of potassium hydroxide, the question arises whether one or more of these enediols may not exist in alkaline solutions of even lower normality than $N/30$ and possibly in solutions of less P_H value than 7; that is, would the molecular mechanism involved in the oxidation of these sugars in an acid solution be in harmony with the concept which postulates the presence of enediols as intermediate substances in reactions of this character?

Experimental Part

The following is an account of the experimental methods used by us in this investigation.

Oxidizing Agent.—Copper acetate was chosen as our oxidizing agent by reason of the following distinct advantages. (*a*) A saturated solution of this salt is acid. (*b*) Since the cupric ion is reduced to the cuprous condition during the progress of the reaction, the cuprous oxide thus formed

[1] (a) De Bruyn and van Ekenstein, *Rec. trav. chim.*, **14**, 156, 203 (1895); (b) **15**, 92 (1896); (c) **16**, 257, 262, 274 (1897); (d) **19**, 1 (1900); (e) **27**, 1 (1908); (f) Wolfrom with Lewis, *J. Am. Chem. Soc.*, **50**, 842 (1928).

[2] (a) Nef, *Ann.*, **403**, 208–213 and note on 239 (1915); (b) compare Powell, *J. Chem. Soc.*, **107**, 1335 (1925), also Armstrong, "The Carbohydrates and the Glucosides," Longmans, Green and Co., **1924**, p. 59; (c) Anderson, *Am. Chem. J.*, **42**, 407 (1909); (d) Spoehr, *ibid.*, **43**, 241 (1910); (e) Upson, *ibid.*, **45**, 458 (1911); (f) Glattfeld, *ibid.*, **50**, 135 (1913); (g) Jensen and Upson, *J. Am. Chem. Soc.*, **47**, 3023 (1925); (h) Gustus with Lewis, *ibid.*, **49**, 1512 (1927).

[3] Evans and co-workers, *ibid.*, **47**, 3086 (1925); **48**, 2666 (1926); **50**, 1496 (1928); Amick, *J. Phys. Chem.*, **31**, 1473 (1927).

[4] Nef, *Ann.*, **403**, 224 (1915).

becomes an accurate measure of the oxygen consumed in the oxidation. (*c*) Although preliminary experiments showed that the rate of oxidation of fructose with copper acetate is greater than that of either glucose or galactose under the same conditions, yet the reaction proceeds slowly enough for the purposes of measurement and it may be stopped at any desired time. (*d*) Certain compounds formed in the oxidation of carbohydrates in acid solutions may escape detection when the same sugars are oxidized in alkaline solutions by reason of either their very rapid rearrangement to other compounds or a further oxidation of the intermediates. (*e*) The unused copper ion is easily removed from solution with hydrogen sulfide.

Oxidation Procedure.—Nine-tenths g. of fructose was placed in a 250-cc. Florence flask containing 25 cc. of a copper acetate solution previously saturated at 50°.

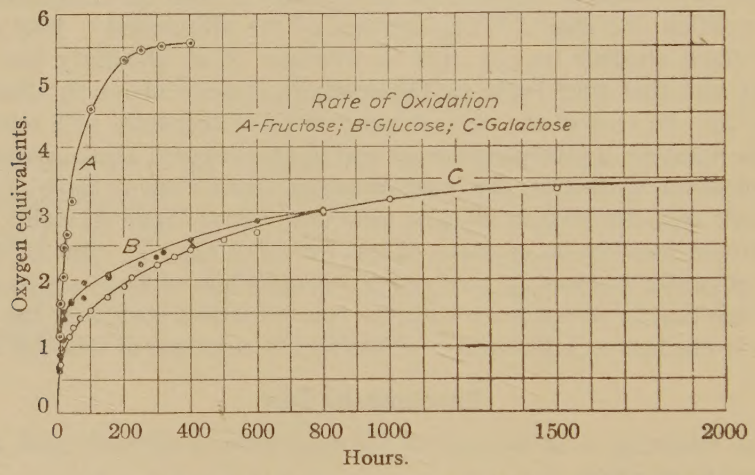


Fig. 1.—Rate of oxidation of fructose compared with glucose and galactose.

To this mixture an excess of copper acetate was added, the amount of it depending on the duration of the experiment. For all experiments of less than one hundred hours 10 g. was added, while 15 g. was sufficient for the longer periods. The flask was closed with a tightly fitting rubber stopper and was then shaken by means of a mechanical agitator in a thermostat kept at 50°.

Determination of Consumed Oxygen.—When the sugar had been oxidized for the desired length of time, the flask was removed and cooled with tap water before removing the stopper in order to avoid the loss of volatile products, for instance, formic acid. The cuprous oxide and unchanged copper acetate were collected in a weighed Gooch crucible, the excess of the acetate being removed by washing the residue with about 50 cc. of cold water. The crucible contents were dried in a vacuum oven at 70–80° without any appreciable oxidation of the cuprous oxide. The rate at which oxygen is used up by fructose, glucose and galactose in solutions of copper acetate at 50° is shown in Fig. 1. The data for glucose and galactose have been taken from the dissertations of Mr. G. C. Strouse and Mr. W. D. Nicoll, respectively. In Fig. 2, it is seen that the relation

between the logarithm of the oxygen equivalents and that of the experimental period is a linear one.

Reaction Products.—Our reaction mixtures were examined for oxalic, formic, glyoxylic and carbonic acids, and glucosone. The percentages of the hexose carbon converted into each of these compounds is expressed as a function of the time in Fig. 3. The number of oxygen equivalents used per gram molecular weight of the hexose was calculated by dividing the weight of the cuprous oxide by the factor 0.716.

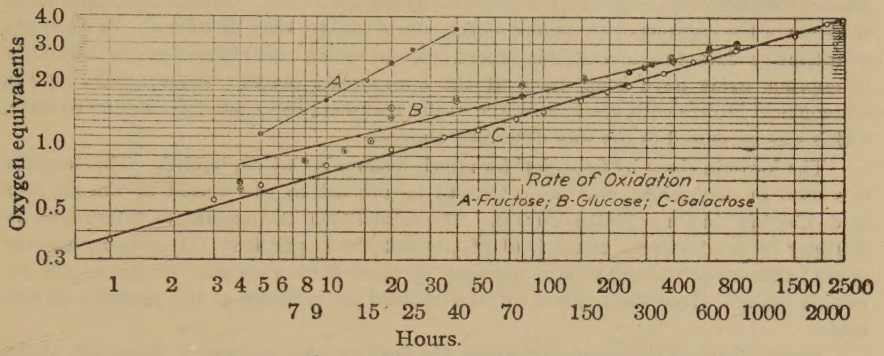


Fig. 2.—Rate of oxidation.

The percentage of the hexose carbon converted to the reaction products for which our mixtures were examined is expressed as a function of the oxygen equivalents in each case in Fig. 4.

Oxalic Acid. (a) **Qualitative Identification.**—When the cuprous oxide and the unused solid copper acetate from the oxidations of longer duration had been filtered and washed with water, a greenish-white, scaly material was found associated with the residual cuprous oxide. From the work of McLeod[5] it was thought that this substance might be copper oxalate, although this investigator states that the cuprous oxide obtained by him contained no copper oxalate. Our suspected oxalate was soluble in dilute ammonium hydroxide solution. Its identity was established by its conversion to the calcium salt, the composition of which was determined by titrating with potassium permanganate

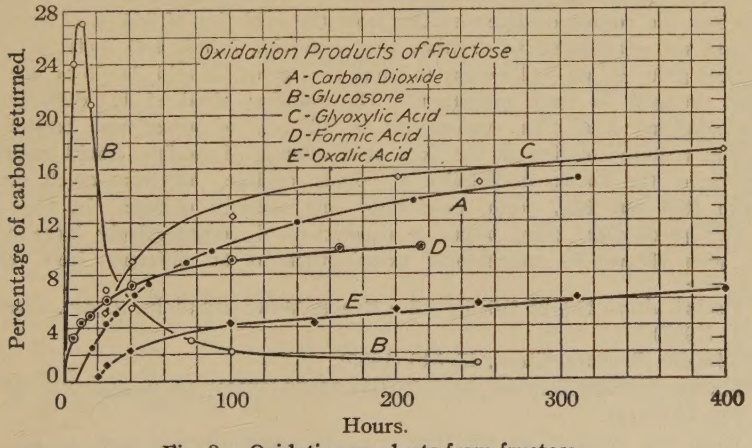


Fig. 3.—Oxidation products from fructose.

[5] McLeod, *Am. Chem. J.*, **37**, 20 (1907).

in the presence of sulfuric acid, and also by converting a weighed portion dried *in vacuo* at 120° into the sulfate. This specimen of calcium oxalate contained 27.3% of calcium, while the monohydrate contains 27.43%. Furthermore, the oxalic acid present in the above ammoniacal solution was also converted into the highly insoluble phenylhydrazine oxalate.[6]

(b) **Quantitative Determination.**—The oxalic acid in these oxidations was determined quantitatively essentially as just described. The cuprous oxide residue was treated with 25 to 30 cc. of dilute ammonium hydroxide (1:19). By this procedure small amounts of the cuprous oxide as well as the cupric oxalate were dissolved. In all of the quantitative data throughout this report, the necessary corrections for the solubility of cuprous oxide have been made at the appropriate places. After acidifying the ammoniacal filtrate with acetic acid, the oxalic acid was precipitated as calcium oxalate, which was measured in the way described above.

The formula given by Schaefer[7] for cupric oxalate is $CuC_2O_4 \cdot {}^1/_2H_2O$. This formula was used in determining the correction to be applied to the weight of the cuprous oxide obtained in our oxidation curves. The solubility of the oxalate, as determined by Schaefer by conductivity methods at 25°, is 0.02364 g. per liter. It was conclusively proved that all of the oxalic acid obtained in these oxidations was present as the insoluble oxalate, since the original filtrates showed no evidence of the presence of this acid.

Formic Acid.—The original filtrate obtained in the procedure described above under "Determination of Consumed Oxygen" was made copper ion-free in the cold by means of hydrogen sulfide. The addition of 1 g. of sodium chloride to this filtrate was found to be fairly effective in preventing the formation of colloidal cupric sulfide. After removing the copper sulfide by suction and washing with water, the filtrate usually had a volume of approximately 150 cc. The presence of formic acid was established by distilling a portion of this copper ion-free filtrate in a vacuum apparatus through which a small amount of natural gas instead of air was allowed to pass. This distillate was examined for formic acid by Fincke's method.[8] It was also found to decolorize dilute alkaline potassium permanganate solutions in the cold. Formic acid was determined quantitatively by using a modification of Jones' well-known method.[9]

Glyoxylic Acid. Qualitative Identification.—(*a*) The solution obtained by the removal of the copper ions from the filtrate from the cuprous oxide, unused solid copper acetate and the copper oxalate resulting from the oxidation of 0.90 g. of fructose for 165 hours was evaporated to dryness, thus removing formic and acetic acids. On theoretical grounds, we had reason to think that glyoxylic acid should be one of the reaction products in these oxidations. The solution obtained by dissolving the residue resulting from this evaporation in 100 cc. of water was treated with a calcium acetate solution in the hope that calcium glyoxalate would precipitate. By reason of the known insolubility of copper oxalate, we were much surprised to learn that our fine, white precipitate was calcium oxalate. In the filtrate from this precipitate, there began a deposition of comparatively large crystals which were shown by microscopic examination to be different from calcium

[6] Fischer, *Ann.*, **190**, 85 (1878). See Evans, Mong and Sinks, *J. Am. Chem. Soc.*, **39**, 1724 (1917).

[7] Schaefer, *Z. anorg. Chem.*, **45**, 310 (1905).

[8] Fincke, *Z. Nahr. Genussm.*, **21**, 1 (1911); **22**, 88 (1911); see Bender, *Bur. of Chem. Bull.*, **162**, 78 (1912).

[9] Jones, *Am. Chem. J.*, **17**, 539 (1887); see Evans, Edgar and Hoff, *J. Am. Chem. Soc.*, **48**, 2667 (1926).

oxalate. A solution obtained by the same procedure from an experiment with 0.90 g. of fructose for 215 hours yielded almost 0.30 g. of these same crystals. When 0.2806 g. of the crystals, previously dried in a desiccator, was fumed with sulfuric acid, 0.1775 g. of calcium sulfate was obtained. This corresponds to 18.62% of calcium, while calcium glyoxalate dihydrate ($Ca(C_2HO_3)_2 \cdot 2H_2O$) contains 18.02% of calcium. In a similar procedure, 0.90 g. of these crystals was obtained from the 200, 300 and

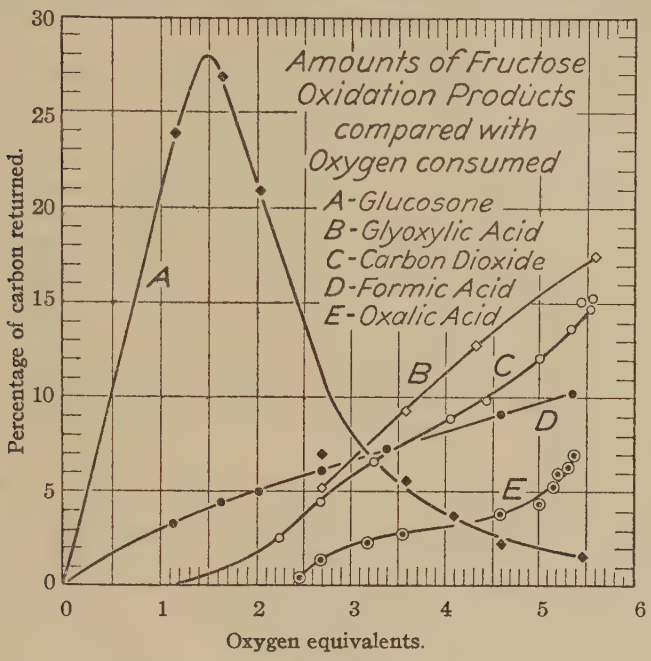


Fig. 4.

400 hour experiments with galactose. On analysis these were found to contain 18.35% of calcium. When the solution from a 200-hour experiment with galactose was treated with barium acetate instead of calcium acetate, a white and fairly crystalline compound was obtained after twenty-four hours' standing. When filtered immediately after precipitation and dried at 100°, this substance had a barium content of 49.9% when fumed with sulfuric acid. An air-dried specimen obtained by slow precipitation was found to contain 47.06% of barium. Anhydrous barium glyoxalate contains 48.48% of barium, the monohydrate 45.59%, and the oxalate monohydrate 56.54%. (*b*) Positive identification of glyoxylic acid was made as follows. After the original filtrates from a 200-hour experiment with fructose and a 300-hour experiment with galactose had been made copper ion-free, they were treated without previous distillation with 3 cc. of phenylhydrazine. If the slow-forming precipitate contained glyoxylic acid phenylhydrazone, then according to Fischer's[10] observations, the latter should be soluble in alkali and be reprecipitated on treatment with hydrochloric acid. When so treated the lemon-yellow colored precipitate was found to melt at 143° (uncorr.). Fischer gave 138°, Pechmann[11] found 143–145°.

[10] Fischer, *Ber.*, **17**, 577 (1884).

[11] Pechmann, *Ber.*, **29**, 2163 (1896).

Qualitative Identification of Glucosone.—When the original filtrates from the glucose and fructose oxidations of five to ten hours' duration were made copper ion-free and were then treated with phenylhydrazine in the cold, glucosazone was immediately precipitated. This behavior corresponds with that of glucosone, which is known to form the osazone in the cold immediately. A control test on glucose and fructose under similar conditions showed that it took several hours for the osazone to precipitate. No galactosone was found in our experiments.

Presence of Unchanged Fructose.—If the glucosazone formed on standing at room temperature for about an hour is filtered, and the resulting filtrate is heated at 100° for an hour, the amount of precipitated glucosazone is so large that it can hardly be accounted for on the ground that it resulted only from the osone still remaining in the solution.

Determination of Glyoxylic Acid, Glucosone and Unchanged Carbohydrate.—The following method was adopted for the estimation of glyoxylic acid, glucosone and unchanged carbohydrate. The solution which had been made copper ion-free was made up to 100 cc. and then treated with 3 cc. of phenylhydrazine. The mixture was allowed to stand at room temperature for three hours. Parallel experiments with fructose and glucose showed no reaction during this time.[12] At the end of this period, the osazone was filtered, allowed to remain in contact with 25 cc. of a saturated sodium carbonate solution for two or three hours, again filtered, washed with water, dried *in vacuo* at 60° and weighed. About 25 cc. of cold benzene was allowed to percolate through the glucosazone in the crucible, which was again dried and weighed. This weight was considered as that of the osazone of glucosone. The sodium carbonate extract was now acidified with hydrochloric acid, which caused a precipitation of any glyoxylic acid phenylhydrazone which might have been present. This yellow-colored precipitate was transferred to a Gooch crucible, dried in a vacuum oven and weighed. From this weight the amount of glyoxylic acid formed in these oxidations was calculated. Fischer[13] states that phenylhydrazine will detect one part of glyoxylic acid in 300 parts of water.

The filtrate from the above osazone was now heated on a boiling water-bath for an hour, after which it was cooled in an ice-bath and filtered. After treatment with sodium carbonate, the residue was washed, dried and weighed as described above, in order to determine the osazone of the unchanged sugar. In all but one case it was found that all of the glyoxylic acid phenylhydrazone was completely removed in the first precipitation with phenylhydrazine. The osazone thus obtained was regarded as that of the unoxidized carbohydrate. The first melting points of the osazones prepared in this way were usually low, 185–190°, but percolation of the crucible contents with two crucible volumes of cold benzene to remove traces of tar resulted in obtaining a pure product, m. p. 206–208°. Previous tests on pure glucosazone had shown that the loss in weight on treatment with cold benzene was well within the limits of error of our method.

With reference to the accuracy of the separation of glucosone and unchanged sugar, our method leaves much to be desired, but every attempt was made to make it relatively accurate. Morrell and Crofts[12,14] found that both glucose and glucosone were precipitated only very incompletely by phenylhydrazine. From this it is to be concluded that our glucosone was not completely precipitated in the cold and that the remainder would be found with the osazone of the unchanged sugar. However, the formation and the partial estimation of the glucosone present as a reaction product, together with the very accurate measure of the oxygen consumed, have aided us very greatly in the

---

[12] See Morrell and Crofts, *J. Chem. Soc.*, **75**, 787 (1899).

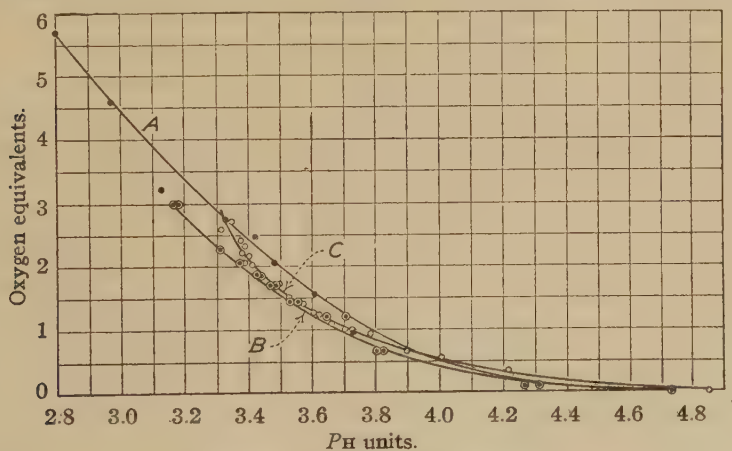
[13] Fischer, *Ber.*, **17**, 577 (1884).

[14] See Fischer, *Ber.*, **21**, 2632 (1888).

interpretation of the mechanism involved in the oxidation of these carbohydrates with copper acetate solutions. Mr. E. F. Schroeder, of this Laboratory, is at present studying the properties of glucosone.

Carbon Dioxide.—During these oxidations gas pressure developed within the reaction flasks. The gas was found to be carbon dioxide. In some cases, especially that of fructose, the pressure became so great that the stoppers were blown from the flasks with considerable force. The apparatus used for the quantitative determination of carbon dioxide consisted of a sufficiently large flask (500–1000 cc.), closed with a stopper fitted with an inlet tube for the admission of carbon dioxide-free natural gas, an outlet tube attached to a small Liebig condenser inclined at an angle of 45° and a mercury seal through which a mechanical stirrer passed. The condenser was connected to two ordinary gas washing bottles arranged in series. These contained barium hydroxide solution. To the outlet of the last wash bottle a soda lime tube was attached. The natural gas used to sweep the carbon dioxide to the absorption train was passed very slowly through a large soda lime tower attached to the inlet tube of the reaction flask. All stoppers were coated with shellac. The reaction flask was immersed in the thermostat. Nine grams of fructose was dissolved in 500 cc. of copper acetate solution saturated at 50° C. The barium carbonate formed in the absorption train was removed by filtration through a porous Alundum crucible, then thoroughly washed with recently boiled water, dried in a vacuum oven and weighed. The crucible was freed from the barium carbonate with dilute acetic acid, carefully washed with water, dried and weighed. The difference between the two weights was regarded as that of barium carbonate.

Hydrogen Ion Concentration.—Preliminary work had shown that the hydrogen electrode could not be used for this work because the platinized platinum electrode soon became coated with copper. However, the quinhydrone electrode gave satisfactory results if the temperature was not too high.[15] We used two general methods in actual practice. (*a*) In one, 3.6 g. of glucose was dissolved in saturated copper acetate



A, fructose; B, glucose; C, galactose.

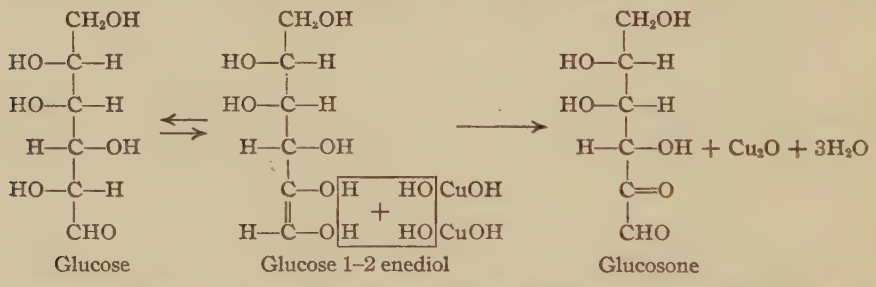
Fig. 5.—Change of hydrogen ion concentration of fructose compared with glucose and galactose.

[15] (a) Mathews, *Trans. Faraday Soc.*, July 6 (1925); (b) Eucken, Jette and LaMer, "Fundamentals of Physical Chemistry," McGraw-Hill Book Company, New York, 1295, p. 396.

solution. The reaction mixture was kept under exactly the same conditions as described for the oxidation procedure. At suitable intervals 5cc. samples were withdrawn and the voltages measured with the saturated calomel electrode and the quinhydrone electrode, using the standard potentiometric method. (*b*) Our second method, employed with fructose and galactose, consisted in using individual reaction mixtures for each reading. In both cases the P_H values were calculated from the equation $P_H = 7.68-(E/0.059)$, in which E is the observed voltage.[16] The change in the hydrogen ion concentration throughout these oxidations is shown in Fig. 5.

Theoretical Part

Glucosone.—It has been pointed out above that one of the main objectives in these experiments was to determine whether the general principles underlying the mechanism of carbohydrate oxidation in alkaline solutions were sufficient to explain the course of such oxidations in acid solutions. Our experimental data show that 23.83% of fructose carbon were recovered from the reaction mixture as glucosone at the end of the first five-hour period. The presence of glucosone in these reactions is easily understood on the assumption that the common 1–2 hexose enediol of glucose and fructose is present in these acidic solutions, and that this enediol is oxidized in accordance with the following reactions.



Such an explanation would regard the enediol as being an acid. In addition to the well-known behavior of these hexoses in alkaline solutions of varying concentrations, the following evidence seems to support this point of view. (*a*) Michael and Rona[17] determined the dissociation constants of some very weak acids, particularly the carbohydrates, and found that the value for fructose was 8.8×10^{-13}, for glucose 6.6×10^{-13} and for galactose 5.3×10^{-13}. (*b*) Powell[18] has shown both by conductivity methods and also by the change of rate in the hydrolysis of ethyl acetate in the presence of fructose and glucose that these carbohydrates form definite compounds with the base, and in the case of sodium hydroxide the reaction takes place in the ratio of one molecule of each. (*c*) Kling[19] found that

[16] LaMer and Rideal, *J. Am. Chem. Soc.*, **46**, 224 (1924).

[17] Michael and Rona, *Biochem. Z.*, **49**, 232–248 (1913); *C. A.*, **7**, 2715 (1913).

[18] Powell, *J. Chem. Soc.*, **107**, 1335 (1915).

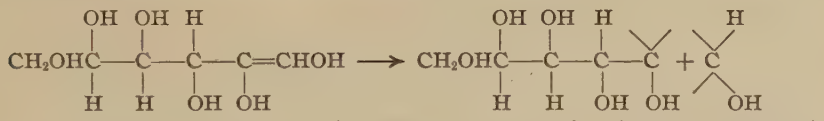
[19] Kling, *Compt. rend.*, **140**, 1256 (1905); see Evans and Hoover, *J. Am. Chem. Soc.*, **44**, 1739 (1922).

acetol gives an acid reaction when dissolved in water and that the acidity returns even after neutralization. Acetol may undergo the following reaction

$$CH_3COCH_2OH \rightleftharpoons CH_3C(OH)=CHOH$$

This observation of Kling with reference to the behavior of acetol toward aqueous solutions of alkalies suggests an analogous behavior in the carbohydrates, especially fructose. (*d*) Wolfrom and Lewis[20] have shown recently that the P_H value of one liter of lime water to which had been added one mole of *d*-glucose is changed from 12.6 to 10.6. In a separate experiment it was established that gluconic acid is not oxidized with copper acetate under the same experimental conditions as those employed with fructose, glucose and galactose. The same observation was made with galactonic acid lactone. Therefore, it is clear that these oxidations proceed by some mechanism other than through the formation of these acids.

Existence of Hydroxymethylene. Formic Acid.—If the hexose-1,2-enediol of these carbohydrates exists in these solutions, the presence of formic acid as an oxidation product shows that the common 1,2-enediol of fructose and glucose are suffering decomposition according to the following equation.



(Fructose-1,2-enediol) (Arabinose—active form) + (Hydroxymethylene)

The active form of the pentose and that of formaldehyde may be oxidized to the pentonic and formic acids, respectively. This interpretation is strongly supported by the recent convincing experiments of Gustus and Lewis on the oxidation of 2,3,4,6-tetramethyl glucose.[2h]

	$CH_2OHCOCOOH$ used, g.	Oxygen equivalents	CO_2	HCOOH
Found	0.193	1.81 (90.5%)	0.008	0.0162 (95%)
Theoretical	.193	2.00	.008	.0171

Carbon Dioxide.—Carbon dioxide was being evolved from fructose at the end of 315 hours. Our quantitative data show that this gas begins to appear as one of the reaction products of fructose at about the same time that the glucosone production has reached a maximum. In view of this fact, we concluded that α-ketogluconic acid was being formed by the direct oxidation of the osone, and that this compound was undergoing decomposition into carbon dioxide and arabinose. Since α-ketogluconic acid is unknown as a stable substance,[21] hydroxypyruvic acid was chosen

[20] Wolfrom with Lewis, *J. Am. Chem. Soc.*, **50**, 842 (1928).

[21] Ruff, *Ber.*, **32**, 2269 (1899).

for our study as being representative of hydroxy-α-keto acids. This acid under the same experimental conditions decomposes with an evolution of carbon dioxide. As the time of oxidation is increased, it is seen from Figs. 3 and 4 that the glucosone gradually is being used up. Ruff found that gluconic acid on being oxidized with bromine was converted into α-ketogluconic acid. In our experiments we found no derivative of α-keto-gluconic acid. By the following special experiments it was conclusively proven that carbon dioxide was not a decomposition product of the following compounds.

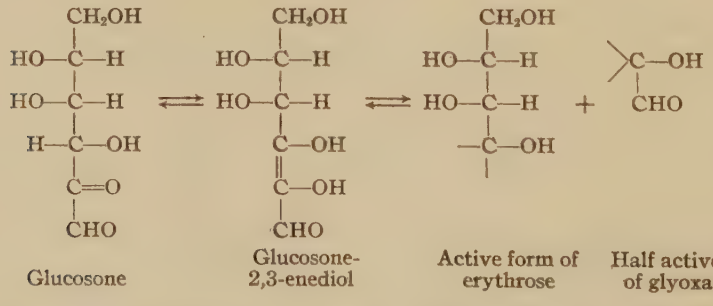
(a) **Galactonic and Gluconic Acids.**—One gram of gluconic acid and of galactonic acid lactone were each treated for one week at 50° with copper acetate. Only a trace of cuprous oxide was formed. This was probably due to some slight impurity. The solid copper acetate disappeared and the solution took on the well-known dark blue color.

(b) **Glyoxal.**—No trace of cuprous oxide was found when glyoxal was treated with copper acetate.

(c) **Glycolic Acid.**—This acid gave the merest trace of reduction.

(d) McLeod made the same kind of observations with formic acid formaldehyde, glycolic acid and oxalic acid.

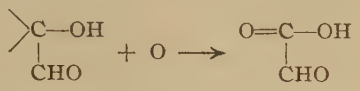
Hexosone-2,3-enediol. Glyoxylic, Oxalic and Glycolic Acids.—(*a*) The hexosones formed in these oxidations may also undergo an enolization. The hexosone-2,3-enediol thus formed may then split at the double bond, thus giving rise to a tetrose and glyoxal. These changes may be represented by the following reactions.



Although Fischer[22] does not record the nature of the insoluble lead compound of glucosone which he prepared by the interaction of lead hydroxide and the hexosone, it is quite probable that it is a lead salt of the hexosone-2,3-enediol. No compounds were formed in our reaction products which indicated that the hexosone-2,3-enediol itself had undergone oxidation with copper acetate. (*b*) In quite the same manner as hydroxymethylene

[22] Fischer, *Ber.*, **21**, 2631 (1888); **22**, 87 (1889).

is oxidized to formic acid, so also is the half active form of glyoxal converted to glyoxylic acid according to the following reaction.



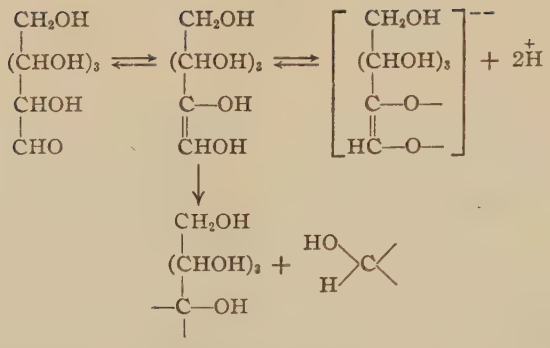
In connection with this point of view concerning the half active form of glyoxal, it is of importance to emphasize that glyoxal itself is unacted upon by copper acetate under the experimental conditions of this work. The oxalic and glycolic acids obtained in these experiments are undoubtedly derived from the glyoxylic acid, a compound which Debus[23] observed would rearrange into these two acids. He also noticed that its calcium salt was converted into calcium oxalate and glycolate in the presence of lime water. Debus furthermore observed that glyoxylic acid does not reduce copper acetate. In solutions of copper acetate containing this acid, we found that copper oxalate was being formed without the formation of any cuprous oxide. With reference to the decomposition of the hexos-one-2,3-enediol, it is of much interest to note that Morrell and Crofts[24] oxidized glucosone with bromine and isolated the erythronic acid so formed as the calcium salt.

Hydrogen Ion Concentration.—It has been pointed out previously that an increase in the alkali normality of aqueous solutions of the hexoses and potassium hydroxide causes a shifting in the equilibrium existing between the three isomeric enediols in the direction of the 3,4-form. The relation existing between the amounts of lactic acid formed from these sugars and the alkali normality favors this point of view, as do also the character and amounts of oxidation products obtained when these aldohexoses are oxidized with alkaline potassium permanganate. The observations of Amick concerning the influence of alkalinity on the copper number of glucose is especially significant in this connection. Therefore, it is clear that an increase in the hydrogen ion concentration of the oxidizing mixtures containing copper acetate should have the opposite tendency and that the degree of ionization of these enediols should be diminished through the mass action effect of the common ion. The recent work of Wolfrom with Lewis on the de-enolization of an equilibrated mixture obtained from 2,3,4,6-tetramethyl glucose supports this point of view.

The equilibrium represented by the following equation would be shifted to the left by an increase in the hydrogen ion concentration.

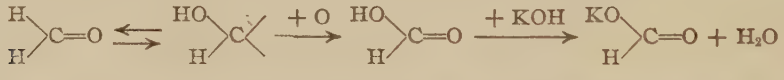
[23] Debus, *Ann.*, **338**, 336 (1905).

[24] Morrell and Crofts, *J. Chem. Soc.*, **81**, 67 (1902).

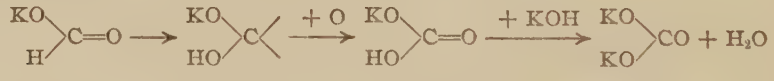


Since this would tend to diminish the concentration of the hexose-1,2-enediol, it follows that the amount of hydroxymethylene would diminish accordingly. This would result in the yield of formic acid being a decreasing function of the time. That this is so is seen in Fig. 11. That the oxidation of the hexose to a hexosone still continues after the cessation of formic acid is evidenced by the fact that glyoxylic and carbonic acids are still forming. In this connection it is of interest to point out that the oxidation of these hexoses with aqueous solutions of copper sulfate proceeds very much more slowly than with copper acetate. Furthermore, when copper acetate solutions are treated with highly ionized acids, the oxidation of the sugars practically stops. We found no evidence for the oxidation of the hexosone-2,3-enediol, since phenylhydrazine did not yield a trihydrazone.

Applying this point of view to the following equation it is seen that alkaline oxidizing agents should yield alkali metal formates.



The conversion of formates into carbonates in alkaline potassium permanganate oxidations must be preceded by a further enolization before oxidation takes place. These changes may be represented by the following equation.



In this general interpretation it is obvious that the ionization of hydroxymethylene after the same manner as the sugar enols might be considered, but we have assumed that the monose is probably oxidized instantly to formic acid. From this discussion one must conclude that the *direct* oxidation of aldehydes in general seems to involve an equilibrium condition in which a certain portion of the aldehyde molecules is in the activated condition. This may be expressed by the following equation

Inactive to oxidation Active to oxidation

In harmony with this point of view with reference to the experiment of Upson and Jensen in which these investigators obtained only 0.5 g. of gluconic acid from 200 g. of glucose is the observation of Amick to the effect that "enolization must occur before oxidation."

Summary

1. Aqueous solutions of fructose have been oxidized with copper acetate at 50° for the purpose of ascertaining whether the general principles underlying the mechanism of carbohydrate oxidation in alkaline solutions are sufficient to explain the course of such oxidations in acid solutions. The advantages in the use of copper acetate are outlined.

2. The reaction mixtures were examined for glucosone, formic, carbonic, glyoxylic and oxalic acids. The presence of glycolic acid was proved qualitatively but it was not measured quantitatively. The quantities of these are expressed both as functions of the time and the oxygen consumed. The oxygen consumed by each sugar is expressed as a function of the time. The hydrogen ion concentration is expressed as a function of the oxygen consumed.

3. The hexose-1,2-enediols are regarded as being oxidized to the hexosones. The glucosone is obtained as the osazone.

4. The formic acid arises from the oxidation of the hydroxymethylene obtained by the decomposition of the hexose-1,2-enediols.

5. It is thought that carbon dioxide arises from the decomposition of the unstable α-ketohexonic acids. Hydroxypyruvic acid loses a molecule of carbon dioxide when oxidized with copper acetate.

6. The hexosones may form hexosone-2,3-enediols, which are thought to decompose into the active form of the tetrose (tetrose methylenol) and glyoxal (half active form). The latter is oxidized to glyoxylic acid, which in part is converted into oxalic and glycolic acids. Glyoxal does not reduce copper acetate solutions, hence it is thought that the glyoxylic acid comes from an activated form (glyoxal-half-methylenol).

7. The increase in the hydrogen ion concentration tends to reverse the equilibrium between the enediols in the direction of suppressing enediol formation. This would lead to a reduction in the formic acid yields with increasing time. The increase in the hydrogen ion concentration would tend to suppress the enolization of the hexosone-2,3-enediol. This harmonizes with the fact that no derivatives were found of 2,3-diketohexoses when the reaction mixtures were treated with phenylhydrazine.

8. The view is expressed that aldehydes exist in two forms, those active toward oxidizing agents and those not. The former are derivatives of methylenols; that is, the hydrogen is bound to oxygen and the carbon is bivalent. In the inactive molecules the carbon is tetravalent. An equilibrium exists between these two forms which may be shifted by a change in the hydrogen ion concentration. The conversion of aldehydes to carbonates in alkaline solutions is explained on this basis.

AUTOBIOGRAPHY

I, Charles Edward Waring, was born in Cincinnati, Ohio, July 10, 1903. I received the first six years of elementary school training in the Public School of Cleves, a suburb of Cincinnati. I entered the Public School of South Charleston, Ohio, in the seventh grade and graduated from the high school there in 1920. My undergraduate college work was taken at Ohio Wesleyan University, Delaware, Ohio, where I received the A.B. degree in 1924. I enrolled in the Graduate School of Ohio State University in 1924, receiving the degree of Master of Arts in 1926, and Doctor of Philosophy in 1927.